S1.13 The external stalk of the F_OF_1-ATPase: Three-dimensional structure of the subunit *b*-dimer as determined by site-specific spin labeling, ESR and molecular modeling

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The structure of the external stalk of the F_oF_1-ATP synthase and its function during the catalytic mechanism remains one of the important and open questions in the field. The external stalk has been proposed to be either a rigid stator that binds and holds F_1 to F_o or an elastic structural element that may be used to store and transmit energy during rotational catalysis. We have employed proteomics, sequence-based structure prediction, molecular modeling and electron spin resonance spectroscopy using site-directed spin labeling to understand the structure and interfacial packing of the *E. coli b*-subunit homodimeric and the *Synechocystis* sp. PCC 6803 *bb′* heterodimeric external stalks of ATP synthases. While comparisons of bacterial, cyanobacterial and plant *b* and *b′* subunits demonstrated little sequence similarity, structure prediction algorithms suggested that all of the compared *b* and *b′*-sequences have extensive heptad repeats. This finding suggests that these proteins may favorably pack as left-handed coiled coils. Molecular modeling followed by energy minimization procedures showed that b_2 from *E. coli* as well as *bb′* from cyanobacterial (*Synechocystis*) ATP synthase were able to pack into low energy left-handed coiled coils. Thirty-eight amino acid substitutions to cysteines in soluble homodimeric *b*-constructs (*E. coli*) were generated and thirty-nine intra- and inter-molecular double cysteine pairs in heterodimeric *bb′*‘ (*Synechocystis*) were prepared that allowed specific introduction of spin labels and the determination of inter- and intra-subunit distances by ESR spectroscopy. Comparison of the inter- and intramolecular interspin distances that we obtained by ESR experiments with the distances that we derived from molecular modeling of both *b*- and *bb′*-dimers as left-handed coiled coils strongly support our proposition that the bacterial *b*-dimer external stalks of ATP synthases indeed form left-handed coiled coils as their low energy structures. Initial ESR experiments using the complete *E. coli* F_oF_1-ATP synthase and where we introduced and spin-labeled specific cysteine pairs in the *b*-dimer of an otherwise cysteine-less enzyme indicate that the inter-subunit packing of the dimer changes during catalytic turnover. This change in the overall winding may be a mechanism by which the *b*-dimer elastically couples the different rotational processes of the enzyme.

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S1.14 Regulatory transitions of subunit epsilon in ATP synthase from thermophilic *Bacillus* PS3

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In bacterial and chloroplast ATP synthase subunit epsilon inhibits ATP hydrolysis; its alpha-helical C-terminal domain is responsible for the inhibition. In *Bacillus* PS3 the C-terminal domain undergoes large conformational changes. In one (inhibitory) conformation the domain is extended along subunit gamma into F_1, while in the contracted (non-inhibitory) conformation this domain is folded as a hairpin close to F_0. We investigated this conformational transition using FRET between Cy3 fluorophore attached to the subunit gamma N-terminus and Cy5 fluorophore attached to the C-terminus of epsilon. Without nucleotides epsilon was in the extended conformation (high FRET) both in F_1 and F_OF_1. ATP induced transition to contracted state (low FRET). ATP concentration dependence of the transition in F_1 and F_OF_1 F_0F_1 was markedly different: the apparent K_d were ~20 and ~200 μM, respectively. ADP had no effect on both conformations, but slowed down the transition induced by ATP. We also studied the role of DELSEED region in subunit beta on epsilon transitions. The negatively charged residues in βDELSEED are important for epsilon inhibitory effect. When the negative residues were substituted to alanines (AALSAAA), much lower ATP concentration induced the transition to contracted state (apparent K_d ~2.5 μM). Moreover, ADP also induced the same transition (although much slower). A scheme for regulatory transition of subunit epsilon is proposed.

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S1.15 The mechanism of inhibition of bovine F_1-ATPase by the inhibitor protein IF_1

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The structure of bovine F_1-ATPase in complex with residues 1–60 of the inhibitor protein, bovine IF_1, has been solved at 2.2 Å resolution. In this structure, the resolved region of I1-60His from residues 8–50 consists of an extended structure from residues 8–13, followed by two α-helices from residues 14–18 and 21–50, linked by a turn from residues 19–20. The long helix of I1-60His (residues 21-55) is 42 Å long. Its binding site in F_1-ATPase is complex. It involves five subunits of the F_1-ATPase, the most significant contributors being subunits β_{DP}, α_{DP} and γ, with more limited contributions from the β_{TP}- and α_E-subunits. In order to understand which residues in IF_1 are important for inhibition, many of the residues in the long helix have been mutated and the effects of the mutations on inhibitory potency have been studied by determination of association and dissociation constants. Most of the binding energy comes from hydrophobic interactions with the β_{DP}-subunit and from an ionic interaction between E30 of IF_1 and R408 in β_{DP}. Many charged residues of IF_1 that are conserved across a wide range of species are not directly involved in binding interactions. Their role appears to be to guide and orient the inhibitor appropriately into its binding site.

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S1.16 Interaction of the peripheral stalk of the bovine ATP synthase with the F_1 domain

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